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Quality and Nutritional Status of Fresh-cut Tomato as Affected by Spraying of Delactosed Whey Permeate Compared to Industrial Washing Treatment.

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Full Title

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Running Head

Shelf-life Extension of Tomatoes by DWP

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Abstract

The aim of this study was to examine the applicability of delactosed whey permeate (DWP) treatment on preserving the quality and antioxidant attributes of fresh-cut tomato. Tomatoes were treated with 3 % DWP by dipping, spraying and a combination of both, stored at 4 °C for 10 days and compared with the industrial standard, chlorine. The combination of dipping and spraying of DWP showed the best results for all the markers tested. The combined treatment of dipping and spraying of DWP significantly lowered total counts (~ 1.0 log cfu/g), yeast and moulds (~ 1.2 log cfu/g), inhibited the loss of firmness (25 %) and reduced POD activity (15 %) of the tomato slices after 10 days compared to the chlorine treatment. Moreover, DWP treated tomatoes maintained significantly ($p<0.05$) higher levels of vitamin C, total phenols (TP) and antioxidant activity (DPPH) than the chlorine treated samples during storage. Sensory scores confirmed that DWP treated tomatoes retained better aroma and texture. Also, the appearance and overall acceptability were higher than chlorine treated tomatoes. Thus DWP treatment has potential to extend the shelf-life of fresh-cut tomatoes.

***Key words*:** Whey permeate; Fresh-cut; Tomato; Shelf-life; Quality, Antioxidants.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a versatile vegetable that is consumed fresh as well as in the form of processed products (Toor & Savage, 2005). It is considered as an important source of dietary antioxidants as it is rich in vitamins, carotenoids and phenolic compounds (Lenucci et al., 2006). Results from epidemiological studies have shown that high consumption of tomato fruit is consistently correlated with a reduced risk of chronic diseases such as cardiovascular disease and certain types of cancer (Sgherri et al., 2008). The increase in the consumers' awareness of the health benefits of fruits and vegetables and the emerging need for convenience due to a fast-paced lifestyle is leading to an increasing demand of fresh-cut fruits and vegetables (Odriozola-Serrano et al., 2008). Retention of the quality and shelf-life of these products during storage is now the interest of the industry and consumers (Camargo et al., 2010). Fresh-cut fruit and vegetables should offer consumers highly nutritious, convenient and healthy food while still maintaining the desired freshness. However, as a result of peeling, cutting and preparation of ready-to-eat fruits and vegetables, a large number of physiological phenomena such as biochemical changes and microbiological spoilage take place and may result in degradation of colour, texture, and flavour. The marketing of fresh-cut vegetables is limited by their short shelf-life due to quick decline in post-processing quality. Chlorinated water (50–200 ppm) is widely used to wash fruits and vegetables as well as fresh-cut produce in order to preserve their quality (Ahmed et al., 2011a, b). However, the possible formation of carcinogenic chlorinated compounds in water (chloramines and trihalomethanes) has called into question the use of chlorine for this purpose (Alegria et al., 2010). In recent years interest is growing in the use of natural products for the preservation of fresh-cut produce. Research and commercial

applications have shown that natural components could replace traditional washing agents (Rojas-Graü et al., 2009). The development of chlorine-free fruit and vegetable products enriched with natural bio-products could contribute greatly to a new and growing market, where the consumers' concerns about their health are met.

Whey permeate is a by-product of the production of whey protein concentrates from cheese whey. The main composition of whey permeate are water, lactose, low molecular peptides and minerals. Whey and whey ultrafiltrated permeate have been proposed to be used as a natural antioxidant in foods (Contreras et al., 2011). Whey proteins and peptides (Lactoferrin, α-lactalbumin and β-lactoglobulin, casein macro peptide, α_{1-} and α_{2-} caseins) exhibit a growing number of biological effects including anti-hypertensive, anti-cancer, hypocholesterolemic, opiodergic, and anti-microbial activities (Román et al., 2011; Yalcin, 2006). They are also a rich source of the amino acids - lysine, leucine, threonine, tryptophan and cysteine. Whey could be a promising natural bio-active alternative to chlorine. Martin-Diana et al. (2006) used acidic whey permeate for washing fresh-cut lettuce and carrots during storage. Whey protein has been found to reduce the enzymatic browning of 'Golden Delicious' apples (Perez-Gago et al., 2006). Coronado et al. (2002) successfully used rosemary extract and whey powder for the oxidative stability of wiener sausages during 10 months frozen storage. Whey is used as a fermentation feedstock for the production of lactic acid, acetic acid, propionic acid, ethanol, and single cell protein, etc (Panesar et al., 2007). However, these applications still do not utilise all the whey produced and new uses for this by-product are needed as the high chemical oxygen demand (COD) (50 kg O_2/ton permeate) of whey makes its disposal a cost-effective and significant pollution problem.

Therefore the aim of this study was to explore the effect of different application methods of delactosed whey permeate treatment on the quality and antioxidant components of fresh-cut tomatoes during storage as an alternative to chlorine.

2. Materials and Methods

2.1. Sampling

Irish vine ripened tomatoes (*Lycopersicon esculentum* L. Mill.) cv. Moneymaker were purchased from a local supermarket (Dunnes Stores). According to the grower, the tomato plants were grown commercially in a greenhouse with a 14 h light period from February until November. The aerial environment of the greenhouse and crop irrigation and nutrition were precisely controlled. The temperature of the greenhouse was 16-21 °C which is optimum for lycopene synthesis in tomato fruits. The tomatoes were then brought to the food processing lab and stored at 4 °C before processing. The experiments were carried out between April to September 2010.

2.2. Preparation of Treatment Solution

Liquid delactosed whey permeate was kindly supplied by Glanbia Ltd. Ingredients, Ireland. Delactosed whey permeate (DWP) was obtained after removal of lactose crystals from whey permeate. The pH for DWP solution was 5.0.

2.3. Processing

Whole tomatoes were rinsed in water prior to washing in order to avoid soil contamination. Washing treatment was performed by applying 3 % DWP solution in three different ways such as dipping, spraying and a combination of both. In case of dipping treatment, whole tomato was dipped in DWP solution (200 g tomatoes/L) for 1 min (with agitation). In case of spraying treatment, the tomatoes were sliced 6 mm in thickness with a commercial slicing machine (Maxwell chase MCT-25, Baltimore

Innovations, UK) and 3 % DWP treatment solution were sprayed over the sliced tomato. In case of the combination of dipping and spraying, tomatoes were treated with 3 % DWP in both ways. For control samples, tomatoes were dipped into 120 ppm chlorine solution (pH 8.0) for 1 min (Delaquis et al., 2004). The tomato slices were then air-dried for 30 mins in RT. Processed tomatoes were then pooled, mixed and ~100 grams placed in a polypropylene tray (180 mm length×130 mm width×25 mm depth) from Sharp Interpack Ltd., UK containing one layer of absorbent paper on the bottom (Fresh-R-Pax absorbent pads, Maxwell Chase Technologies, Atlanta). The principal ingredient in fresh-R-Pax absorbent pads is food grade sodium carboxymethyl cellulose (CMC), a common ingredient in ice-cream, sauces, low-fat foods, etc. The trays were then packed in bags (200 × 320 mm) of 35 µm oriented polypropylene film (OPP) with permeability at 23 °C and 90 % RH of 3.3×10^{-12} mol/s/m^2/Pa for O_2 (Amcor Flexibles Europe-Brighouse, United Kingdom). The packages were then heat-sealed under atmospheric conditions and stored at 4 °C for 10 days (Ahmed et al., 2011a).

2.4. Markers Analysis of Fresh-cut Tomato

Different quality (headspace gas composition, colour, pH and firmness), sensorial and nutritional markers (ascorbic acid, lycopene, total phenols, antioxidant activity as measured by DPPH), enzymatic activity (POD and PME) and microbial enumerations (total aerobic counts and yeast and moulds) of fresh-cut tomato were monitored throughout the 10 days of storage at 4 °C.

2.4.1. Quality Markers

2.4.1.1. Headspace Gas Composition

Changes in O_2 and CO_2 concentration of the headspaces of the fresh-cut tomatoes packages were monitored during the shelf-life of fresh-cut tomatoes. A Gaspace analyser (Systech Instruments, UK) was used to monitor O_2 and CO_2 levels. Gas extractions were performed with a hypodermic needle, inserted through an adhesive septum previously fixed to the bags, at a flow rate of 150 ml/min for 10 sec. Three bags per treatment were monitored for each experiment and all bags for other analyses were checked before analysis (Ahmed et al., 2011b).

2.4.1.2. Colour

For colour analysis each piece of tomato in the storage pack was analysed individually to minimise the variability of the product. Colour was quantified using a Colour Quest XE colorimeter (HunterLab, Northants, UK). A tomato slice was placed directly on the colorimeter sensor (3.5 cm of diameter) and measured. 20 – 30 measurements were taken per treatment and day. The L* parameter (lightness index scale) range from 0 (black) to 100 (white). The a* parameter measures the degree of red (+a*) or green (-a*) colour and the b* parameter measures the degree of yellow (+b*) or blue (-b*) colour. The CIE L* a* b* parameters were converted to Hue (arctan b*/a*) and Chroma $(a^{*2}+b^{*2})^{1/2}$.

2.4.1.3. pH

For pH value 10 g of tomato tissue were blended for 2 min. The pH was measured at room temperature using an Orion research pH-meter, UK.

2.4.1.4. Firmness

Four measurements were made on each slice, two in the outer pericarp and two in the radial pericarp, applying the force in the axial direction. The force necessary to cause a deformation of 3 mm with a speed of 0.02 mm/s was recorded using a an Instron

texture analyser (Instron 4302 Universal Testing Machine, Canton MA, USA), with a 3.5 mm diameter flat faced cylindrical probe. Only the central slice in the stack was used in the analyses. The firmness measurement was performed immediately after removing the slice from the storage chamber (at storage temperature). Data were analysed with the Instron series IX software for Windows.

2.4.2. Enzymatic Activity

2.4.2.1. Browning-related Enzyme - Peroxidase (POD)

POD enzyme was assayed in homogenates that were prepared as follows: 10 g of tomato puree was placed in a 100 ml beaker in a 1:2 (w:v) ratio with 0.5 M phosphate buffer, pH 6.5, containing 50 g/l polyvinylpyrrolidone. Then homogenisation was carried out twice with an Ultra-Turrax T-25 tissue homogeniser at 4 °C and 20,500 rpm, for 1 min each time with a break of 3 min between homogenisations to avoid excess heating of the sample. The homogenate was centrifuged at 12,720 g for 30 min at 4 °C. It was then filtered through Whatman no. 4 filter paper. The resulting crude extract was used without further purification. All the extracts were kept at 4 °C in the dark and used immediately (within 1 hr). POD activity was assayed spectrophotometrically by a modified method based on Martin-Diana et al. (2006). The reaction mixture contained 0.2 ml of extract and 2.7 ml of 0.05 M phosphate buffer, pH 6.5, containing 1.85 ml of hydrogen peroxide (1.5 %, v/v) as oxidant and 3.7 ml of p-phenylendiamine as hydrogen donor. The oxidation of p-phenylendiamine was monitored at 485 nm and 25 °C. A unit of enzyme activity was defined as an increase of 0.1 absorbance units per minute.

2.4.2.2. Texture-related Enzyme - Pectin Methyl Esterase (PME)

PME activity was measured using the method described by Yoo et al. (2009). Ten grams of tomato puree was diluted in an extraction solution (0.2 M sodium phosphate buffer, pH 7.5 containing 1 M sodium chloride and 10 mM dithiothreitol) and homogenised at 4 °C for 2 min at 5,500 rpm. The macerate was incubated at 4 °C for 30 min with agitation and centrifuged at 12,720 g for 30 min at 4 °C. It was then filtered through Whatman no. 4 filter paper. One ml of this extract was mixed with 40 ml of substrate solution (1 % pectin). The solution was adjusted to pH 7.0 with 1.0 M NaOH, and the pH of the solution was readjusted to pH 7.5 with 0.05 M NaOH. After the pH reached 7.5; 0.2 ml of 0.05 N NaOH was added. The time required to return to pH 7.5 was recorded. Activity was quantified as carboxyl groups formed by the hydrolysis of methyl esters of pectin and was measured tritrimetrically using a pH electrode to monitor the production of H^+. The enzymatic activity was expressed as:

$$PME = \frac{0.2[mL]NaOH * 0.05[Mol \cdot L^{-1}]NaOH \cdot X[mL] \cdot 10^3[mMol \cdot Mol^{-1}] \cdot 10^3[L \cdot mL^{-1}]}{Y[mL] \cdot Z[g] \cdot time[\min]} \quad (1)$$

Where, X = total volume (ml) extracted, Y = volume used (1 ml) in the assay, Z = sample used in the assay (10 g). Three macerates per treatment and day were prepared. Triplicates of the enzymatic activity were analysed.

2.4.3. Sensory Analysis

A panel of 12 judges aged 20 - 35 years (postgraduate students of the School of Food Science and Environmental Health, DIT) was trained in discriminate evaluation of fresh-cut tomato. Panellists were required to score changes in fresh appearance, texture, aroma and general acceptability. Before starting the sensory experiments, panellists were familiarised with the product and scoring methods. This consisted of demonstration exercises involving examination of fresh-cut tomatoes at different levels of deterioration and agreeing appropriate scores. After becoming familiar with

the test facilities and scoring regime, they were invited to score samples. This procedure was repeated several times until a level of consistency in scoring was obtained. Fresh appearance, texture, aroma and general acceptability of tomato samples were scored on a scale of 1 to 9, where a score of one indicated a product of very poor quality, etc. (Ferreira et al., 2008). The evaluation was carried out in the sensory evaluation laboratory. Products were placed in plastic cups with lid, on a white surface and judges were isolated from each-other in a booth in an odour-free environment. Samples were presented in randomised order to minimise possible sequence influence. The results of the sensory analysis were reported as means of three separate trials. Data were analysed using Compusense® Five software (Release 4.4, Ontario, Canada).

2.4.4. Nutritional Markers

2.4.4.1. Ascorbic Acid

The ascorbic acid content in fresh-cut tomatoes was analysed by high performance liquid chromatography (HPLC) following the method described by Lee & Castle (2001) with a slight modification. A 25-ml of 6 % meta-phosphoric acid (pH 3.0) was added to 2.5 g of tomato samples. The sample was homogenised for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogeniser. Then the sample was shaken with a Gyrotory Shaker G-2 (USA) for 2 hrs at 150 rpm and centrifuged for 15 min at 3,000 g at 4 °C (Sanio MSE Mistral 3000ii, UK). Following centrifugation, 10 ml of the supernatant was filtered through PTFE syringe filters (pore size 0.45 μm, Phenomenex, UK) and stored at - 20 ºC in foil covered plastic test tubes for further analysis by HPLC. The analysis of ascorbic acid content was performed with Waters 600 Satellite HPLC, with a reverse phase analytical polymeric C_{18} column (150 × 4.6 mm, 5 μm) (Waters, Ireland) with a UV-tunable absorbance detector (Waters 486) at

230 nm. Ten µl of the sample was injected. An isocratic mobile phase of 25 mM monobasic potassium phosphate (pH 3.0) with a flow rate of 1.0 ml/min was used. Five concentrations of ascorbic acid standard in 6 % meta-phosphoric acid in the range 10 - 50 µg/ml were injected.

2.4.4.2. Lycopene

Ten grams of tomato samples were weighed and transferred into a 100 ml beaker (wrapped with aluminium foil). A 50-ml volume of hexane-acetone-ethanol solution (2:1:1 v/v/v) containing 2.5 % BHT was added to solubilise the lycopene (Shi & Le Maguer, 2000). Following this the samples were homogenised with an Ultra-Turrax T-25 tissue homogeniser for 1 min at 20,500 rpm. The samples were then shaken with a Gyrotory Shaker G-2 (USA) for 2 hrs at 150 rpm followed by 10 ml of distilled water was added and stirred for additional 10 min. The polar and non-polar layers were separated, and the upper hexane layer was collected and filtered through a 0.45 µm PVDF membrane filter. It was transferred to a new 15 ml aluminium wrapped test tubes and kept at - 80 °C for analysis. The analysis of lycopene was performed with Waters 600 Satellite HPLC, with a reverse phase analytical polymeric C_{18} column (150 × 4.6mm, 5 µm) (Waters, Ireland) with a UV tunable absorbance detector (Waters 486) for spectrometric peak. The lycopene peaks were identified at 475 nm. An isocratic mobile phase of methyl t-butyl ether/methanol/ethyl acetate (40:50:10, v/v) with a flow rate of 1 ml/min was used. The column temperature and mobile phase was maintained at 25 ºC. Analyses were performed under dim light to prevent sample degradation by photo-oxidation. Three concentrations of lycopene standard in the range 0.01 - 0.03 mg/ml were injected.

2.4.4.3. Total Phenols

For extraction of total phenol content 1.25 g of tomato sample was weighed and 25 ml of methanol was added. Following this the sample was homogenised in a 50 ml tube with an Ultra-Turrax T-25 tissue homogeniser for 1 min at 24,000 rpm. The samples were then thoroughly mixed with a vortex mixer (V400 Multitude Vortexer, Alpha laboratories) for 2 hrs at 150 rpm. Then it was centrifuged for 15 min at 3,000 g using a Sanyo MSE Mistral 3000i, UK. Following centrifugation, 10 ml samples of the supernatant were filtered through PTFE syringe filters (pore size 0.45 μm, Phenomenex, UK). Finally the extracts were stored at – 20 °C in foil covered plastic test tubes for further analysis. Total phenol content of tomatoes was determined using the Folin-Ciocalteu method (Singleton et al., 1999). In a 1.5 ml Eppendorf tube, 100 μl of appropriately diluted methanolic extract, 100 μl of MeOH and 100 μl of FC reagent were added and vortexed. After exactly 1 min, 700 μl of sodium carbonate (20 %) was added, and the mixture was vortexed and allowed to stand at room temperature in the dark for 20 min. Then the tubes were centrifuged at 12,720 g for 3 min. The absorbance of the supernatant was read at 735 nm in 1 ml plastic cuvettes. The blank was MeOH. Each sample of the three batches was measured in triplicate. Results were expressed as mg/L Gallic acid equivalents (GAE).

2.4.4.4. Antioxidant Activity Test - 2, 2-Diphenyl-l-picrylhydrazyl Radical Scavenging Capacity Assay (DPPH)

DPPH scavenging activity assay was performed as per the method described by Sanchez-Moreno (2002) with a slight modification. For extraction, 1.25 g of tomato sample was weighed and 25 ml of methanol was added to it. Following this the sample was homogenised in a 50 ml tube with an Ultra-Turrax T-25 tissue homogeniser for 1 min at 24,000 rpm. The sample was then thoroughly mixed with a vortex mixer (V400 Multitude Vortexer, Alpha laboratories) for 2 hrs at 150 rpm.

Then the sample was centrifuged for 15 min at 3,000 rpm using a Sanyo MSE Mistral 3,000i, UK. Following centrifugation, 10 ml samples of the supernatant were filtered through PTFE syringe filters (pore size 0.45 μm, Phenomenex, UK). Finally the extracts were stored at - 20 °C in foil covered plastic test tubes for further analysis. In a 1.5-ml Eppendorf tube 500 μl of appropriately diluted methanolic extract and 500 μl DPPH Reagent were added and vortexed. After that they were kept for 30 min in dark. The absorbance of the supernatant was read at 515 nm in 1 ml plastic cuvettes. Each sample of the three batches was measured in triplicate.

2.4.5. Microbial Analyses

Microbiology analyses were carried out on the samples before and after the treatment at regular intervals through the storage period. 25 g of tomatoes were blended in 225 ml of peptone water (Scharlau Chemie, S.A., Barcelona, Spain) with a Stomacher circulator homogeniser (VWR, Dublin, Ireland). Enumeration and differentiation of total aerobic counts were quantified at 30 ºC in plate count agar (Scharlau Chemie, S.A., Barcelona, Spain) over 72 hrs. Yeast and moulds were quantified at 25 ºC in potato dextrose agar (Scharlau Chemie, S.A., Barcelona, Spain) over 72 hrs. The results were expressed as log colony forming units per gram (cfu/g).

2.4.6. Statistical Analysis

Data were analysed by multivariate analysis of variance (MANOVA) using Statgraphics software (version: Centurium XV; Statistical Graphics Co., Rockville, USA) for different washing treatments. Analysis of variance one-way (ANOVA) was used to analyse each treatment over storage. In the case of significant differences the LSD range test ($p<0.05$) was used.

3. Results and Discussion

3.1. Quality Markers

3.1.1. Headspace Gas Composition

Fig. 1 illustrates the changes of headspace gas (O_2 and CO_2) composition inside the fresh-cut tomato packages during the 10 days of storage. Both the oxygen and carbon dioxide gas compositions significantly ($p<0.05$) changed over storage. The oxygen gas decreased and the carbon dioxide gas increased throughout storage, as expected. Oxygen decreased from atmospheric levels (21 % - packaging conditions) to values around 15 % by day 10. A sharp increase in carbon dioxide was observed during storage, from 1.0 % to reaching values around 4.5 % at day 10. The final concentrations for both gases in the fresh-cut tomato packages stored at 4 ºC were between the tolerance limits for this commodity which are neither lower than 10 % for oxygen nor higher than 15 % for carbon dioxide (Villanueva et al., 2005). Similar gas concentration levels at day 10 were also described by Artés et al. (1999) using passive MAP where the fresh-cut tomatoes retained good quality. Gil et al. (2002) also found this increase in CO_2 after cutting of fresh tomatoes. There was no significant ($p<0.05$) difference among the control and the DWP treatments for headspace gas composition as the changing pattern of the gases was the same over time.

3.1.2. Colour

Colour is one of the most important factors in the perception of the quality of fresh-cut fruits and vegetables. In the present study, fresh-cut tomatoes showed a significant decrease ($p<0.05$) in luminosity during storage (Table 1). Lana et al. (2006) also found a similar trend. There were significant ($p<0.05$) differences in L* values between samples treated with DWP by spraying and samples treated with chlorine. The other two DWP treatments did not show significant difference with the chlorine

335 treatment. The parameters a* and b* were not significantly ($p>0.05$) affected by the
336 DWP treatment. The parameter a* increased significantly ($p<0.05$) during storage.
337 The a* value is an important parameter for red colour development and the degree of
338 ripening in tomato. Lana et al. (2006) also found the increase of a* values during
339 storage. However, the parameter b* decreased during storage for all treatments. Hue
340 and Chroma also decreased during storage and the decrease was more prominent in
341 samples treated with chlorine than samples treated with DWP, though not significant.
342 Hue is negatively correlated with the maturity of tomato. Hue decreases as tomato
343 matures during storage.

344 *3.1.3. pH*

345 Significantly ($p<0.05$) higher pH was observed for the control samples (chlorine) than
346 samples treated with DWP (Fig. 2A). At day 10 the lowest pH appeared in samples
347 washed with DWP by a combination of dipping and spraying method, followed by
348 spraying only. Tomatoes treated with DWP by dipping had the highest pH among the
349 three DWP treatments, though the difference with the tomatoes treated with DWP by
350 spraying was not significant ($p>0.05$). The pH increased significantly ($p<0.05$) over
351 storage in all the samples. This is in agreement with Artés et al. (1999). The increase
352 in pH during storage might be associated with bacterial growth (Rico et al., 2007).

353 *3.1.4. Texture*

354 Texture (firmness) decreased significantly ($p<0.05$) during storage for all treatments
355 (Fig. 2B). These instrumental result correlated with the sensory panel's texture scores
356 (Fig. 3). All three DWP treated samples maintained significantly ($p<0.05$) better
357 texture than chlorine treated samples. Significant differences ($p<0.05$) among DWP
358 treatments were also observed. Samples treated with DWP by a combination of

dipping and spraying method maintained significantly higher texture throughout the storage than samples treated with DWP dipping and DWP spraying. There was no significant ($p>0.05$) difference between the samples treated with DWP dipping and DWP spraying. The presence of calcium in whey permeates might help to maintain the firmness of tomato during storage (Evans et al., 2010). Different calcium salts have been used for firmness improvement of fresh fruits and vegetables. Calcium carbonate and calcium citrate are the main calcium salts added to foods in order to enhance the nutritional value. Calcium chloride has been widely used as preservative and firming agent in the fruits and vegetables industry for whole and fresh-cut commodities (Chardonnet et al., 2003).

3.2. Enzymatic Activity

3.2.1. Browning-related Enzyme- Peroxidase (POD)

The data for POD activity of fresh-cut tomato showed that samples treated with DWP had significantly ($p<0.05$) lower activity compared to chlorine treated samples (Fig. 2C). Significant differences ($p<0.05$) in POD activity were observed among the DWP treatments. Samples treated with DWP by a combination of dipping and spraying method and DWP spraying showed significantly ($p<0.05$) lower POD activity than samples treated with DWP dipping throughout the storage. The decreased activity of POD in DWP treated samples might be associated with the potential antioxidant activity of the whey permeate used (Peña-Ramos & Xiong, 2003). A significant ($p<0.05$) increase in the activity in all the samples was observed during storage regardless of the treatments, although showing little fluctuations over storage. The initial increase at day 3 might be due to mechanical stress during minimal processing (Cantos et al., 2001). The depletion of antioxidants at day 10 might be attributed to the sharp increase of POD activity in whey treated samples in the period of day 7 to

10. Edible composite coatings prepared from whey protein concentrate and beeswax with ascorbic acid or 0.5 % cystine reduced the enzymatic browning of 'Golden Delicious' apples (Perez-Gago et al., 2006).

3.2.2. Texture-related Enzyme- Pectin Methyl Esterase (PME)

Significant differences ($p<0.05$) were observed in PME activity among the samples treated with DWP and chlorine (Fig. 2D). Samples treated with chlorine showed significantly ($p<0.05$) lower PME activity than DWP treated samples. There was no significant difference ($p>0.05$) among the DWP treated samples. PME activity increased significantly ($p<0.05$) during storage for all treatments. Other authors have attributed that the variability of intrinsic factors and the pre- and postharvest factors can affect enzyme activity, vitamin content, etc of the samples (Yoo et al., 2009). Similar behaviour has been observed for certain browning related enzymes (Perez-Gago et al., 2006).

3.3. Sensory Analysis

Significant differences ($p<0.05$) for fresh appearance, aroma, texture and general acceptability scores were observed between samples treated with DWP and chlorine (Fig. 3). DWP treated fresh-cut tomatoes scored significantly higher ($p<0.05$) than chlorine treated samples. Among the samples treated with DWP, the combination of dipping and spraying scored the highest, followed by the spraying only. Lowest scores of fresh appearance, aroma, texture and general acceptability were observed in samples treated with DWP by dipping only among the three DWP treated samples. This was in agreement with most of the physico-chemical markers of fresh-cut tomatoes studied in the current research. All the attributes evaluated (such as, texture, aroma, first impression, general acceptability) decreased significantly ($p<0.05$) during

storage which is associated with a loss of quality. However, the values at the end of the storage (10 days) were still above the acceptability threshold of 5 for all the attributes scored (Ferreira et al., 2008). The non-hypoxic O_2 and CO_2 concentration in the packages might have helped to maintain acceptable levels of colour and aroma (Aguayo et al., 2006). Nykänen et al. (1998) analysed the effect of nisin-whey permeate washing solutions on total counts and sensory characteristics in rainbow trout. They found that nisin-whey treatment caused no negative effect on sensory attributes.

3.4. Nutritional Markers

3.4.1. Ascorbic Acid

Ascorbic acid decreased significantly ($p<0.05$) during 10 days of storage for all treatments (from 19 mg/100 g FW to 15 mg/100 g FW). This result is in agreement with Toor & Savage (2005). Significant differences ($p<0.05$) were observed in ascorbic acid content among the samples treated with DWP and chlorine (Fig. 4A). Samples treated with chlorine retained the lowest ascorbic acid over storage. Samples treated with DWP by a combination of dipping and spraying had significantly ($p<0.05$) higher ascorbic acid content than the other two DWP treated samples after 10 days of storage. However, there was no significant difference ($p>0.05$) between the samples treated with DWP by dipping and by spraying. Ascorbic acid contributes 28–38 % to the antioxidant activity, while the remaining activity is mainly due to phenolics in tomatoes (Toor & Savage, 2005). Phenolic substances have been reported to have a protective effect on the ascorbic acid.

3.4.2. Lycopene

The average amount of lycopene in the tomato samples was 5.7 mg/100 g FW. The treatments did not show a significant effect ($p<0.05$) on the lycopene concentration of the samples during storage (Fig. 4B). Samples treated with chlorine retained the lowest lycopene over storage. The highest lycopene content was observed in samples treated with DWP by a combination of dipping and spraying, though the difference was not significant ($p>0.05$). However, storage time had significant effect ($p<0.05$) on the tomato samples. The lycopene content increased during storage. This is because fruits biosynthesise carotenoids during ripening throughout storage time (Odriozola-Serrano et al., 2008). On the other hand, Shi & Le Maguer (2000) observed that carotenoids are susceptible to oxidation in the presence of light, oxygen and low pH. Therefore, the increase in the lycopene concentration during 10 days of storage might be due to the biosynthesis of lycopene induced by ripening and the low oxidation of this carotenoid as a result of low availability of O_2 in the package headspace.

3.4.3. Total Phenols

The total phenol content of tomato samples differed significantly over storage time (Fig. 4C). Chlorine treated samples showed significantly ($p<0.05$) lower phenolic content than DWP treated samples. All three DWP treated tomato samples maintained similar level of phenolic content during storage. The total phenol content decreased significantly ($p<0.05$) during storage irrespective of treatments. This was in accordance with other studies (Toor & Savage, 2005; Gil et al., 2002). The decrease was slow until day 3. After this, all samples demonstrated a rapid decrease in the total phenolic content. Chlorine treated samples decreased the most to a value of around 19.8 mg GAE/100 g FW from 21.0 mg GAE/100 g FW over 10 days of storage. Phenolics are the major antioxidant compounds in plant extracts. Toor & Savage

(2005) reported that phenolic compounds might contribute 60 to 70% antioxidant activity of tomato extracts.

3.4.4. Antioxidant Activity Test - 2, 2-Diphenyl-1-picrylhydrazyl Radical Scavenging Capacity Assay (DPPH)

The antioxidant capacity as measured by DPPH radical scavenging activity differed significantly ($p<0.05$) between treatments (Fig. 4D). All three whey permeate treated samples showed significantly ($p<0.05$) higher DPPH reduction than chlorine treated samples. The higher antioxidant activity of whey permeates treated samples could be associated with the intrinsic antioxidant activity of whey permeates (Ahmed et al., 2011a). Whey permeates might have also helped to retain the antioxidant activity of tomato slices. These results could be related to the total phenolic content of the samples since the samples containing higher phenolic content exhibited stronger DPPH reduction and vice versa. There was no significant difference ($p>0.05$) between the samples treated with DWP by a combination of dipping and spraying and spraying only. Samples treated with DWP by dipping only had the lowest DPPH reduction among the three DWP treatments. On the other hand, the antioxidant capacity of fresh-cut tomatoes depleted with storage time irrespective of the treatments. The antioxidant activity was reduced on an average ~ 4 % over 10 days of storage at 4 °C.

3.5. Microbial Enumerations

3.5.1. Total Aerobic Counts

Fresh-cut tomatoes stored at 4 °C had initial loads of total aerobic counts approximately ~ 4.5 log cfu/g. The DWP treated samples had significant difference ($p<0.05$) of bacterial growth to those treated with chlorine (Fig. 5A). Samples treated with DWP by a combination of dipping and spraying showed the highest reduction (~

1.0 log cfu/g) of total counts than samples treated with chlorine after 10 days of storage. Samples treated with DWP by dipping only and spraying only also showed significantly ($p<0.05$) better reduction in total aerobic counts (~ 0.6 log cfu/g) than samples treated with chlorine after 10 days of storage. In fresh-cut tomatoes, total counts increased during storage for all the washing treatments. This increase was more obvious between days 7 and 10. The values of DWP treated samples at the end of the storage were lower than the recommended 10^8 cfu/g for consumer consumption of fresh-cut vegetables (Alegria et al., 2010).

3.5.2. Yeast and Moulds

The initial loads of yeast and moulds of fresh-cut tomatoes stored at 4 °C was approximately 4 log cfu/g. This result was in agreement with the finding of Prakash et al. (2002) for diced tomato. Chlorine treated samples showed the highest yeast and moulds growth (~ 7.0 log cfu/g, day 10). DWP treated tomatoes showed significantly ($p<0.05$) lower counts than chlorine treated samples (Fig. 5B). Samples treated with DWP by a combination of dipping and spraying showed the highest reduction (~ 1.2 log cfu/g) of yeast and moulds counts than samples treated with chlorine after 10 days of storage. DWP treated samples by spraying also showed significantly ($p<0.05$) better reduction in yeast and moulds counts (~ 0.8 log cfu/g) than samples treated with chlorine after 10 days of storage. Yeast and moulds increased significantly ($p<0.05$) for all the washing treatments with storage time.

The presence of antimicrobial peptides in the whey permeate might contribute to its antimicrobial capacity (Clare & Swaisgood, 2003). Antimicrobial peptides have been identified from whey protein hydrolysates. The most studied are the lactoferrins, α_{S1}-casein and α_{S2}-casein (Mccann et al., 2006). These antimicrobial peptides act against different gram-positive and gram-negative bacteria (*Escherichia, Helicobacter,*

Listeria, Salmonella and *Staphylococcus*), yeasts and filamentous fungi (Rizzello et al., 2008; Fitzgerald & Murray, 2006). The amphipathic nature of these peptides presumably underlies their biological activities which enables them to associate with lipid membranes and disrupt normal membrane functions of bacteria. The mechanism of action has been investigated for whey antimicrobial peptides by Saint-Sauveur et al. (2008).

4. Conclusion

The results showed that the use of delactosed whey permeate (DWP) is a viable alternative to chlorine in controlling the microbiota associated with the quality deterioration of fresh-cut tomatoes, since the growth of total aerobic counts and yeasts and moulds were substantially inhibited by its application. Moreover, DWP treated samples retained the antioxidant compounds better during storage than the chlorine treated samples. The three application methods of DWP differed significantly for extending the shelf-life of fresh-cut tomatoes. The combination of dipping and spraying of DWP showed the best results for all the markers tested. Further research on antimicrobial and antioxidant properties of DWP is recommended.

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648

649 **Figure Captions**

650 **Fig. 1.** Effect of treatments on headspace gas composition O_2 (A) and CO_2 (B) in
651 fresh-cut tomato packages over 10 days storage at 4 °C. Points designated on any
652 curve by different letters are significantly different ($p<0.05$). Lower case letters are
653 used for comparisons during storage and upper case letters for treatment comparisons.
654 Three independent trials were carried out in triplicate.

655 **Fig. 2.** Effect of DWP and chlorine treatments on pH (A), texture (B), POD (C) and
656 PME (D) in fresh-cut tomato packages over 10 days storage at 4 °C. Points designated
657 on any curve by different letters are significantly different ($p<0.05$). Lower case
658 letters are used for comparisons during storage and upper case letters for treatment
659 comparisons. Three independent trials were carried out in triplicate.

660 **Fig. 3.** Sensory evaluation of fresh-cut tomatoes packaged and stored for 10 days at 4
661 °C and treated with DWP and chlorine.

662 **Fig. 4.** Ascorbic acid (**A**), lycopene (**B**), total phenols (**C**) and antioxidant activity -
663 DPPH (**D**) in fresh-cut tomatoes treated with DWP and chlorine during the 10 days of
664 storage at 4 °C. Points designated on any curve by different letters are significantly
665 different ($p<0.05$). Lowercase letters are used for comparisons during storage and
666 uppercase letters for treatment comparisons. Three independent trials were carried out
667 in triplicate.

668 **Fig. 5.** Effect of washing treatments on total aerobic counts (A) and yeast and moulds
669 (B) during 10 days storage of fresh-cut tomato at 4 °C. Points designated on any curve
670 by different letters are significantly different ($p<0.05$). Lowercase letters are used for
671 comparisons during storage and uppercase letters for treatment comparisons. Three
672 independent trials were carried out in triplicate.

673

Fig. 1

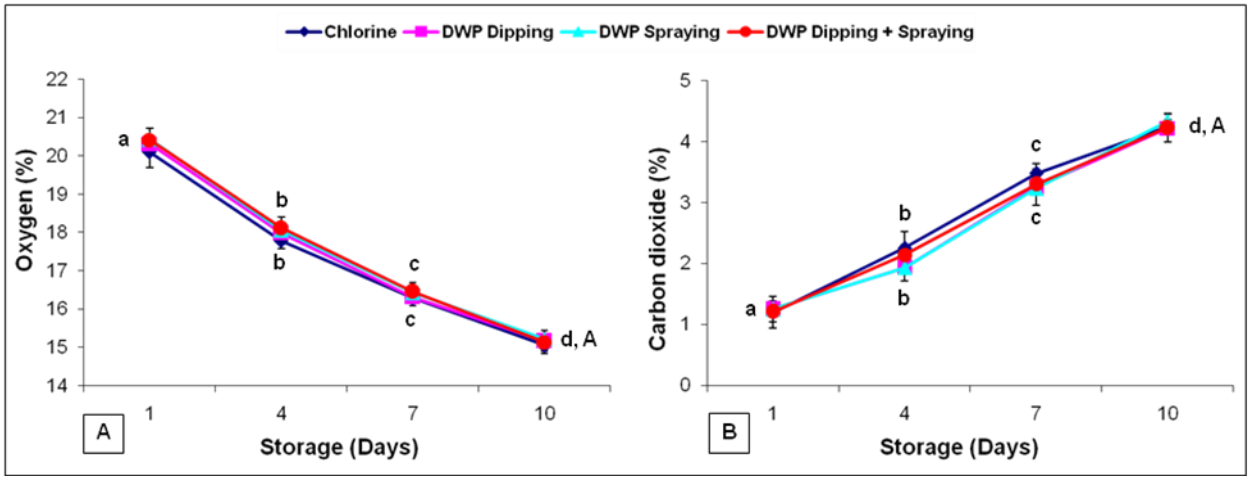

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Fig. 2

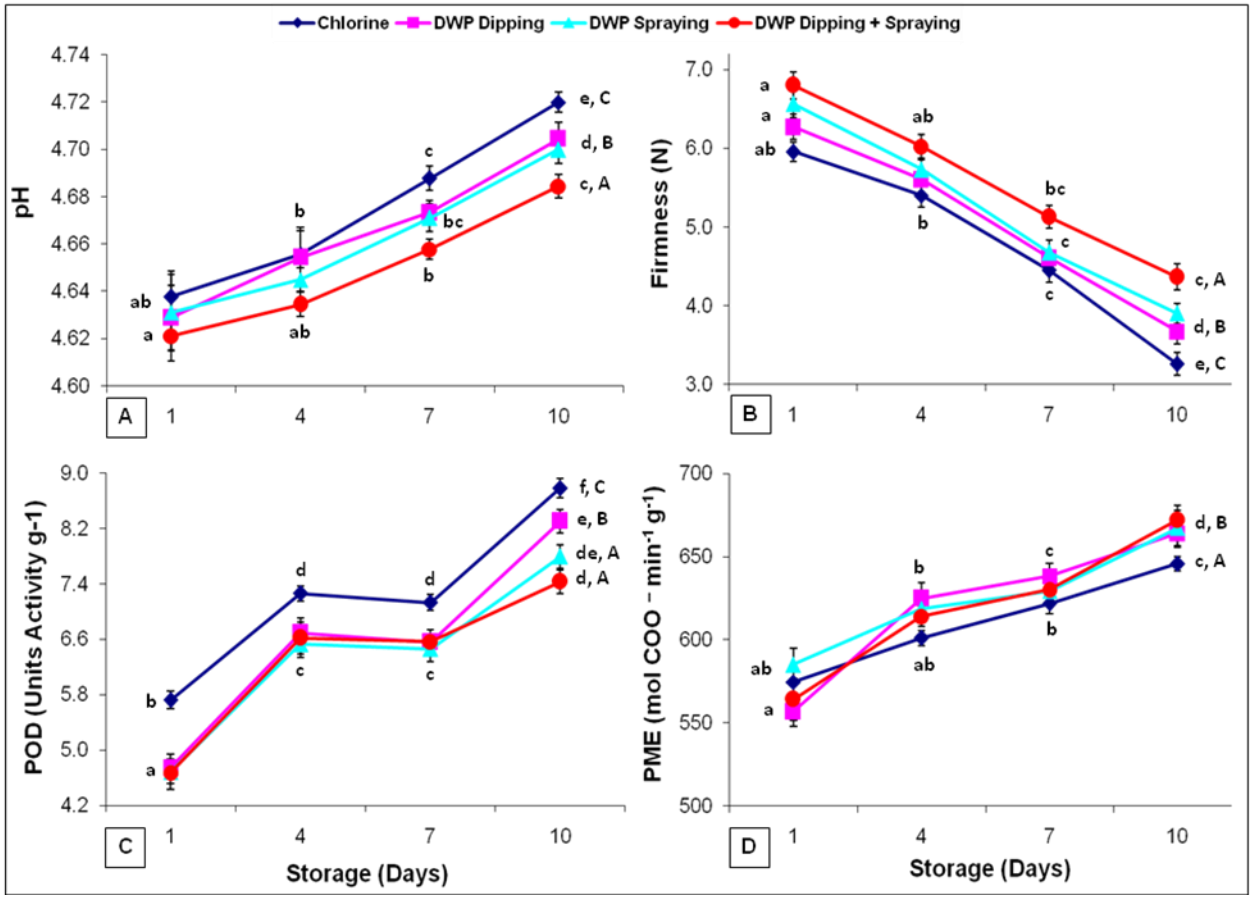

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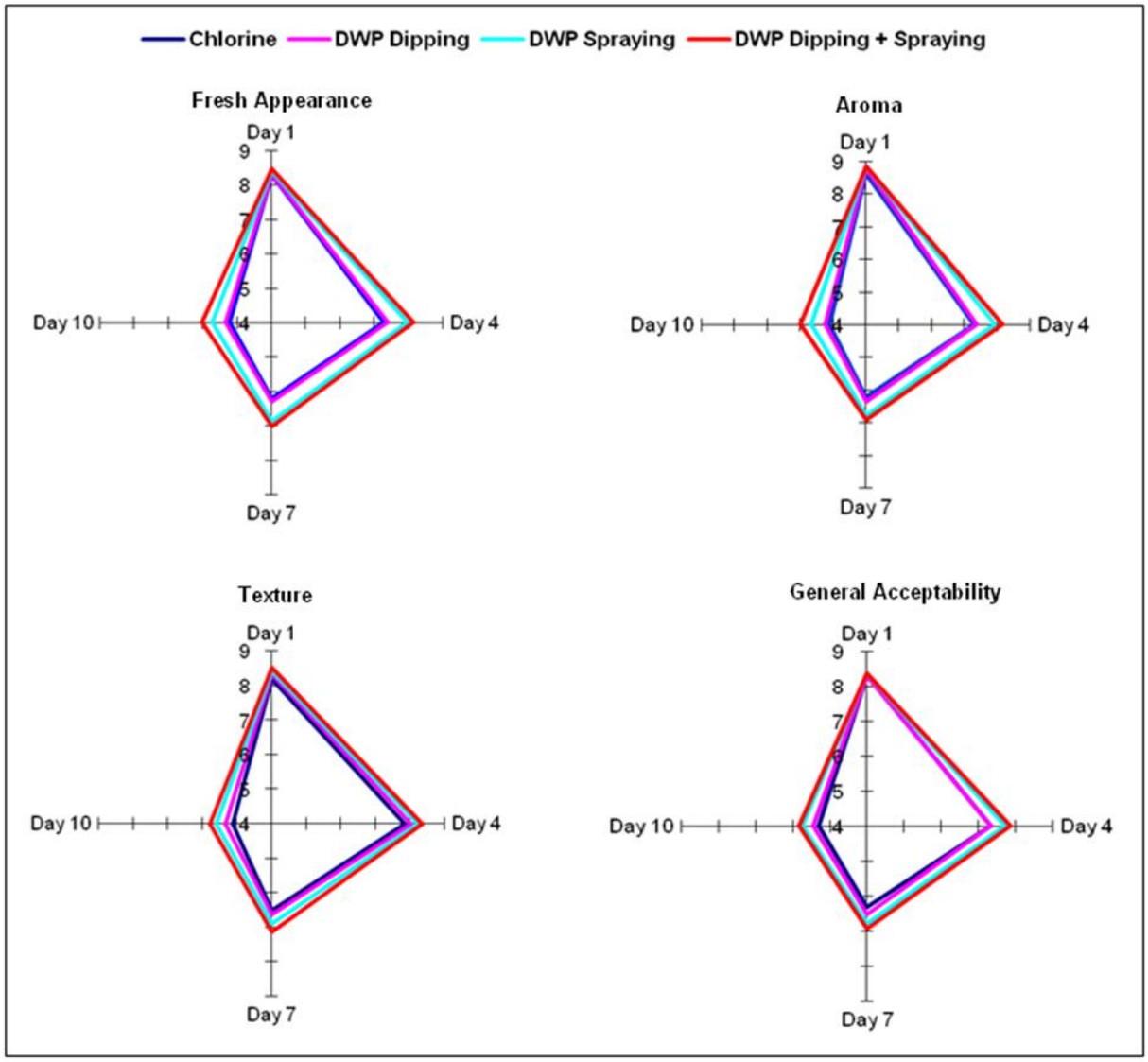
Fig. 3


Chlorine
DWP Dipping
DWP Spraying
DWP Dipping + Spraying
Fresh Appearance
Aroma
Texture
General Acceptability
Day 1
Day 4
Day 7
Day 10
9
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4

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Fig. 4

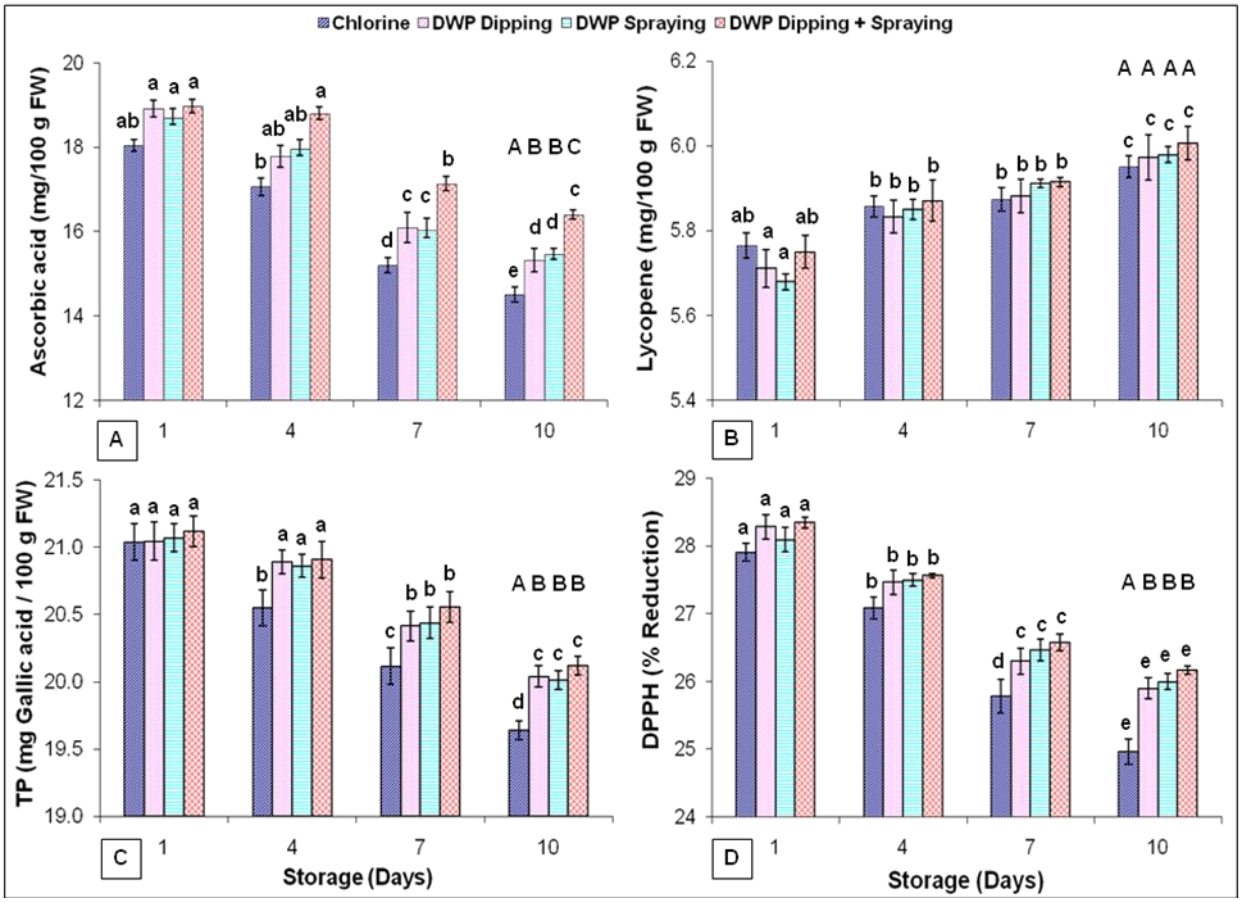

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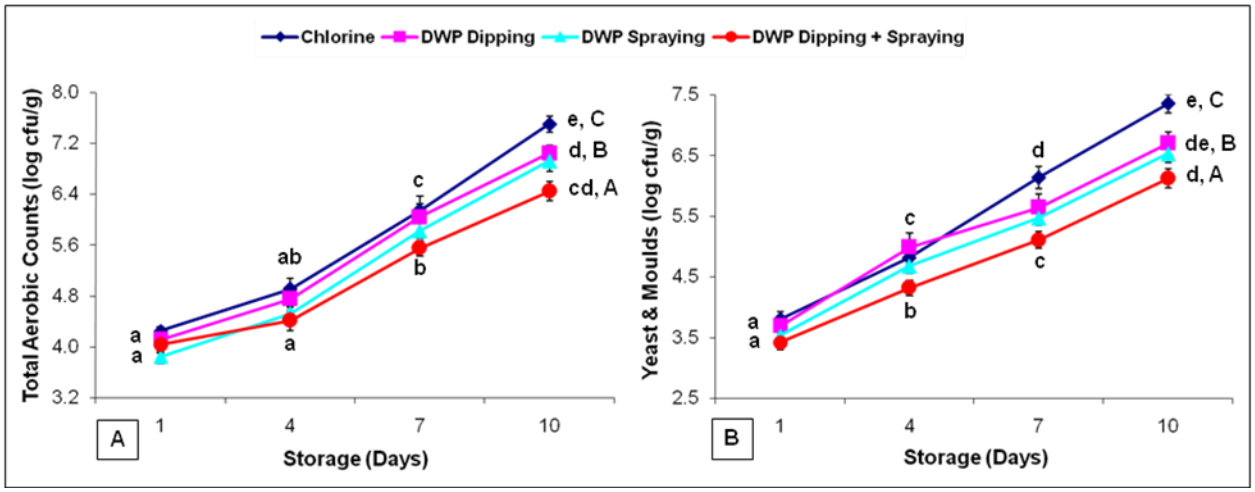
Fig. 5



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